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14. ABSTRACT

It is estimated that 10% to 20% of warfighters who have served in Iraq and Afghanistan have PTSD 1-4. An important limitation of these estimates is the reliance on self-report screening measures and clinical interviews to make the diagnosis of PTSD. These methods are subject to a number of biases, including underreporting of PTSD symptoms because of stigma of mental illness and concerns about adverse effects on careers, and exaggeration of symptoms in those seeking compensation for service- connected disability. Development of biomarkers of PTSD is critical for DOD and VA as objective indicators of PTSD for use in post-deployment medical screening, treatment selection, treatment outcome monitoring, disability evaluations, and for informing novel targets for treatment development.

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None provided.

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Biomarkers for PTSD

Year 4

Submitted 7/23/2013

INTRODUCTION:

It is estimated that 10% to 20% of warfighters who have served in Iraq and Afghanistan have PTSD [1-4]. An important limitation of these estimates is the reliance on self-report screening measures and clinical interviews to make the diagnosis of PTSD. These methods are subject to a number of biases, including underreporting of PTSD symptoms because of stigma of mental illness and concerns about adverse effects on careers, and exaggeration of symptoms in those seeking compensation for service- connected disability[5]. Development of biomarkers of PTSD is critical for DOD and VA as objective indicators of PTSD for use in post-deployment medical screening, treatment selection, treatment outcome monitoring, disability evaluations, and for informing novel targets for treatment development. Additionally, biomarkers hold great potential for explaining and mitigating the associations between war zone-related PTSD and physical health problems, including cardiovascular and metabolic disorders [6-10]. In order to address this critical gap we will perform a pilot study to determine feasibility for larger scale biomarker identification and biomarker informed intervention studies by carefully examining 200 OIF/OEF warfighters through an extensive biological protocol. The first phase will pilot the integration of methods across five leading research laboratories and identify the most promising biomarkers in preparation for larger scale studies. Given the sample size for the pilot and large number of biomarkers of interest, we will specify a limited set of biomarkers for hypothesis testing. It is predicted that compared with controls the PTSD group will have smaller dentate/CA3 hippocampal subfield volumes, lower ambient cortisol levels, and greater cortisol suppression following dexamethasone administration. It is also predicted that lower neuropeptide Y levels will be associated with smaller Dentate/CA3 volumes, and that APO E4 polymorphisms will be associated with smaller Dentate/CA3 volumes.

Goal:

The major goals of this study are:

(1) Advance the diagnosis of PTSD by developing objective biological indicators of the disorder that are not subject to the under and over reporting biases associated with self-report and clinical interview measures;
(2) Advance the understanding of the pathogenesis of PTSD, for example identifying susceptibility genes, neuroendocrine and neurochemical markers and the underlying neurocircuitry of PTSD;
(3) Identify novel biomarker correlates of risk and resilience factors that explain individual differences in the onset, severity, course and impact on functioning of war zone PTSD; and
(4) Identify novel panel of sensitive and specific biomarkers for PTSD and conduct validation and replication studies on these markers.

PROJECT ACCOMPLISHMENTS:

The Biomarkers for PTSD study is in the implementation phase. In year 4 of the grant we accomplished several milestones and goals. Our success in meeting these goals is detailed below.

1. IRB

IRB protocols underwent amendments to reflect new provisions (including the PCL in the initial screening process, inclusion of 6 external specialized labs working with Dr. Wolkowitz for the metabolism core, and increasing the sample size to include an additional 80 female veterans). We received IRB approvals from NYU, Bronx VA, MSSM and UCSF. We streamlined the approval process by informing the DOD IRB of all changes during the continuation review.

We submitted an application to the NYU full board for the annual continuation review and we received approval from the NYU IRB on October 23. The study protocol is now renewed until October 22, 2013. We submitted an application to NIH/NIMH to extend the expiration date of the Certificate of Confidentiality. The application for extension was approved and the certificate was extended until 12/31/2016. We submitted an Application to Human Research Protection Office (HRPO) for the annual continuation review and received approval until 22 October 2013. We also received annual continuation renewal with CTSI until 11/15/2013

2. Personnel

Dr. Steve Hamilton left his position at UCSF to practice medicine and he is not involved in research. Dr. Kerry Ressler from Emory University is now the PI for the genetic core on this study.

Kerry Ressler, MD, PhD, a Howard Hughes Medical Institute investigator who was elected as a member of the 2012 class of the Institute of Medicine, researches the molecular and cellular mechanisms of fear learning and the process of extinction of fear in mouse models and in humans with fear-related disorders. The primary objective of the work in the Ressler lab is to use the power of molecular genetics to understand the molecular biology, neural circuitry and behavioral biology of fear and extinction of fear in mouse models.
Dr. Ressler is also a practicing psychiatrist with an interest in translational and clinical research on fear-based psychiatric disorders. His clinical psychiatry research, conducted at Grady Memorial Hospital, focuses on post-traumatic stress disorder (PTSD), and he is a leader in the area of genetic underpinnings of fear and anxiety disorders. Ressler hopes that by understanding how fear works in the mammalian brain in the laboratory, it will improve understanding of and provide translational treatments and possibly prevention for fear-based disorders, such as PTSD, phobic disorders and panic disorder. Dr. Ressler has been the principal or co-principal investigator on more than 10 grants from the National Institutes of Health (NIH) as well as multiple foundation grants, including the Brain and Behavior Research Foundation and the Burroughs Wellcome Fund.

3. Communication & Reporting

The team continued to engage in bi-weekly communication meetings via teleconference to ensure the successful and timely execution of the Implementation Phase. Calls took place between the PIs and investigators at each site (SF VAMC, UCSF, Mt Sinai, Bronx VA and NYU). Meetings addressed safety issues, clinical questions, strategies for improving subject recruitment and enrollment, strategies for maximizing participation in Visits 2-4, and ensuring that participants moved through all stages of the study quickly and efficiently (in order to avoid attrition).

The clinical team, under the supervision of Dr. Henn-Haase, conducted weekly calibration meetings across sites to establish clinical consensus in scoring the frequency and intensity of symptoms on the CAPS and clinical

assessment. Each discrepancy from the evaluation of participants was resolved by group consensus during these meetings.

Core PIs participated via Web-Ex in an internal quarterly meeting on May 3rd & May 8th 2012 presenting preliminary findings from each core.

PIs participated in the Systems of Biology quarterly meetings and provided preliminary data on demographics of the recruited sample, data from diagnostic clinical evaluations, self-reports and neurocognitive data. Preliminary biomarkers data on neuroimaging, genetics, endocrine and metabolic biomarkers were also presented during the May 14, 2012 and July 1-2, 2013 meeting.

Dr. Marmar also presented at the Systems Biology in Progress Reporting (IPR) meeting on February 20th, 2013 at Fort Detrick, Maryland. The data included project progress including the Male and Female Biomarkers for PTSD studies. The report included an overview of demographics of the recruited sample, data from the diagnostic clinical evaluation, self-reports and neurocognitive data and areas of statistical significance. Dr. Marmar and the core PIs also presented biomarkers findings on neuroimaging markers, genetic markers, multi-omics markers, endocrine and metabolic markers. Dr. Marmar also presented preliminary data from the Voicemarkers for PTSD study.

4. Outreach to Partner Organizations for Research Participant Recruitment:

We have developed an extensive network of 76 partner organizations for research participant recruitment.

American Veterans for Equal Rights
Art Therapy Outreach Center NY
Black Veterans for Social Justice
Bronx Vet Center
Brooklyn Vet Center
Dutchess County Division of Veterans Services
Ed Thompson Veterans Center
Fort Hamilton
Hope for the Warriors
Institute for Community Living
Institute for Family Health
Integral Yoga Institute
Iraq and Afghanistan Veterans of America
Iyengar Yoga Association

JBFCS Home Again
Maimonides Sleep Disorder Clinic
Mayor's Office of Veteran's Affairs
Montford Point Marines Association, Inc.
New Era Veterans
New Jersey War related Illness and Injury Study Center (WRIISC)
New York Institute of Technology
New York Public Library/Single Stop USA Veteran Hub
Project Renewal
Project TORCH
PROVE (Project for Return and Opportunity in Veterans Education)
Queens Vet Center
Rutgers Anxiety Disorders Clinic Veteran PTSD Support Group
Samaritan Village Veterans Program
Staten Island Vet Center
The Doe Fund
Times Square Church Military Ministry
VA NY Harbor HCS
Veteran Stand Down
Veterans Writing Workshop
Warrior Writers
Workforce 1
Yoga Warriors
Baruch College
Bergen County Community College
Berkeley College
Borough of Manhattan Community College
Bronx Community College
Brooklyn College Student Veterans Club
Brooklyn College
College of Staten Island
Columbia University Counseling and Psychological Services
Columbia University MilVets
Fairleigh Dickinson University
Fairleigh Dickinson University Center for Psychological Services
Hostos Community College
Hudson County Community College
Hunter College
Hunter College Student Veterans Club
John Jay Armed Forces/Veterans Association
Kingsborough Community College
LaGuardia Community College
Lehman College
Medgar Evers Community College
Mercer County Community College
Mercy College
Middlesex County College
Middlesex County College Student Veterans Club

Nassau Community College
New Jersey City University
Norwalk Community College
Pace University Student Veterans Club
Passaic County Community College
Queens College
Queensborough Community College
Rutgers Student Veterans Club
Rutgers University
The City College of New York
The City College of New York Veterans Club
Touro College
NAMI Family-to-Family 12-Week Course for Veterans

5. Research Participant Recruitment and Enrollment

Recruitment has continued, through year, at a high rate. During this reporting period, we clinically assessed 465 veterans, of these 171 met full eligibility criteria; 81 met criteria for combat related PTSD and 90 were found negative for combat related PTSD. For the 171 study participants, we completed clinical assessment measures, entered and managed their data, conducted biomarker acquisition procedures (imaging, genetics, endocrinology, metabolism, and proteomics), and delivered materials to cores.

6. Data Management

All Clinical Assessment data from the baseline interview, self-report and neurocognitive measures for all study participants were completed and entered as digital data directly into the study secure SQL database server. Data from all cores is also shared with NYU and saved into a single database. The lead biostatistician at NYU analyzed and scored all the Clinical and Self Report measures on a cohort of 52 PTSD positive and 52 controls who are matched on age, gender and ethnicity. The data was saved on the NYU server and shared with all the cores. Each core is able to access to the data via VPN log in. A variable description and dictionary was also developed and shared with all the cores.

7. Acquisition of Study procedures

All study procedures including blood draw procedures and MRI imaging are completely operational.

Please see the table below for the total number of study procedures completed to date:

Biomarkers For PTSD	
BCI Evaluation	
Clinical Assessment	465
Eligible by Evaluation	171
PTSD+	81
PTSD-	90
Cognitive Testing	
Completed	146
PTSD+	66
PTSD-	80
Self-Report Questionnaire	
Completed	160
PTSD+	72
PTSD-	88
Visit 2 Blood Draw (1)	
Completed	155
PTSD+	76
PTSD-	79
Visit 3 Blood Draw (2)	
Completed	150
PTSD+	73
PTSD-	77
MRI	
Completed	148
PTSD+	65
PTSD-	83

8. Shipment of Material to cores

Shipments of Blood samples were transferred to all collaborating sites, Integrative Systems Biology Laboratory: Mouse Models of PTSD (Principal Investigator Dr. Jett), and Institute for Systems Biology (ISB): Genetics, Metabolomics (Principal Investigator Dr. Hood), Genetics Core at UCSF (PI: Dr. Steve Hamilton), and to the Metabolism Core at UCSF (PI: Dr. Owen Wolkowitz).

Data transfer from NYU to the imaging core at UCSF is running smoothly and Q & A procedures indicate high quality of data collection. All scans were processed

through freeSurfer v5.1. Manual Hippocampal Subfield Marking on scans was completed on 99 subjects.

9. Data Sharing Agreements:

NYU and Bronx VA teams are worked with Privacy Officers and Offices of Industrial Liaison/Technology Transfer (OIL) to execute Material Transfer Agreements (MTAs) with 6 specialized laboratories that will conduct analysis on assays for the metabolism core. Four of these MTAs were excuted. The cores are now working on executing MTAs between NYU, the Bronx VA/MSSM and Emory University to be able to ship samples to Dr. Ressler's lab.

10. Standard Operating Procedure (SOP) Manuals

All cores collecting specimens are working on refining detailed SOP manuals for all procedures in this study. The finalized SOPs for the Human studies will be stored collectively in the Datacube

11. Publication and Dissemination Committee

The group developed a publication and dissemination committee. The committee developed guidelines for reviewing proposals and manuscripts before they are published. These guidelines were shared with the investigators on this study. All investigators will submit a manuscript pre-proposal form to be reviewed by the publication committee before the manuscript is written.

List of Members of the Publication Policy:

1) Charles Marmar (Chair)
2) Marti Jett
3) Rachel Yehuda
4) Mike Weiner
5) Owen Wolkowitz
6) Frank Doyle

Members of the publication committee will not contribute to the writing or be authors merely by being members of the committee, although any member of the committee may participate in any writing team.

12. Publications & Dissemination of Data:

During this reporting period, one manuscript was published in the Neuroscience Letters. The article is cited as:

Yan X, Brown AD, Lazar M, Cressman VL, Henn-Haase C, Neylan TC, Shalev A, Wolkowitz OM, Hamilton SP, Yehuda R, Sodickson DK, Weiner MW, Marmar CR. Spontaneous brain activity in combat related PTSD. Neurosci Lett 547: 1-5

Manuscripts in Preparation

Yehuda R, Flory J, Henn-Haase C, Desarnaud F, Daskalakis NP, Lehrner A, Koch E, Zhang TY, Makotkine I, Marmar CR, Meaney MJ. Lower methylation of glucocorticoid receptor gene promoter 1F in peripheral blood of veterans suffering from post-traumatic stress disorder. (In prep)

Yan X, Brown AD, Lazar M, Henn-Haase C, Yehuda R, Flory JD, Neylan TC, Wolkowitz OM, Hamilton S, Sodickson DK, Weiner MW, Marmar CR. Precuneal and amygdala functional connectivity in warzone-related PTSD. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. Elevated rated of metabolic syndrome in male combat veterans with post-traumatic stress disorder: Associations with symptom severity. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. Serum metabolomics analysis in male combat veterans with post-traumatic stress disorder. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. Peripheral blood markers of cellular aging in male combat veterans with post-traumatic stress disorder. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. Hematopoietic and immune cell alterations in male combat veterans with post-traumatic stress disorder. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. Serum BDNF concentrations in male combat veterans with post-traumatic stress disorder. (In prep)

Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Henn-Haase C, Abu-Amara D, Reus V, Coy M, Dhabhar S, Marmar CR. High pro-inflammatory cytokine levels in male combat veterans with post-traumatic stress disorder. Biological Psychiatry (Under Internal Review)

Conferences:

Dr. Marmar and all core PIs presented a 2 hour symposium at the annual American Psychiatric Association meeting on May 22, 2013 in San Francisco. The presentation included an overview of the study, data from the clinical and self-report data, data from the following cores:

1. Neurocognitive
2. Endocrine
3. Metabolism
4. Genetics
5. Multi-omics

13. Preliminary Analysis

A summary of findings on the 52 PTSD cases and 52 controls that are matched on age, gender and ethnicity is listed below:

The presented findings are considered preliminary and exploratory due to modest sample size and multiple hypothesis testing. Findings will be validated in a new, replication study.

- There are a significant group differences in both current and past major depression
- There are significant group differences in alcohol abuse and dependence.
- Controlling for education, there are significant differences between groups in Working Memory/attention using Digit Span, Spatial Addition (IQ is a trend toward significance)
- PTSD is associated with increased markers of allostatic load and with immune system dysregulation
- Metabolic dysregulation is directly associated with symptom severity
- BDNF concentrations are higher in PTSD positive subjects and are directly correlated with PTSD symptom severity
- Telomere shortening (indicative of cell aging) is directly associated with overall psychiatric symptom severity in PTSD positive subjects
- Combat exposed OIF/OEF veterans with PTSD had cortical thinning in the rostral anterior cingulate and insula compared to combat exposed OIF/OEF veterans without PTSD.

- The structural abnormalities in the rostral anterior cingulate were associated with abnormalities at the network level indicating an increased interaction with other brain regions that together with abnormal interactions in non a priori regions without significant thinning/volume loss ultimately resulted in a different global organization in PTSD.

- GR gene methylation is promising, and seems less sensitive to physiological health factors such as obesity.

- PTSD is associated with increased markers of the cardio-metabolic syndrome, increased pro-inflammatory cytokines and dysfunctional senescent natural killer cells

- Metabolomic analysis suggests mitochondrial dysfunction and inefficient intracellular energy generation, consistent with preclinical models of PTSD

- We have found molecular panels to objectively provide "Yes/No" assessment to supplement psychological evaluations using a) methylated gene-specific loci, b) miRNA.

KEY RESEARCH ACCOMPLISHMENTS:

- Obtained IRB continuation approvals across all sites and the DOD.

- Continued outreach efforts and networking with various veterans and community organizations. IRB approved recruitment material (brochures, flyers and advertisements) were distributed at job fairs, colleges, VA Medical Centers and veterans' organizations.

- Enrolled 171 OIF/OEF veterans and completed clinical assessments, self-report measures, and neurocognitive testing for these participants.

- Study team participated in weekly study meetings, quarterly internal meetings and Systems Biology meetings.

- Entered, cleaned, and scored all data into a centralized database and ran reports for data analysis.

- Completed biomarkers study procedures for eligible participants including blood draws, MRIs and urine collection.

- Completed a number of shipments of blood samples from JJPVAMC to UCSF (Metabolism & Genetic cores). Neuroimaging data was transferred successfully from NYU to UCSF.

- Executed Material Transfer Agreements between JJPVAMC, NYU and outside specialized labs collaborating with the Metabolism Core at UCSF.
- Prepared analysis on a 52/52 cohort
- Participated in a 3 hour symposium at the Annual American Psychiatric Association (APA) conference.

REPORTABLE OUTCOMES:

- The major development during the timeframe of this annual report for this project is that the implementation phase has been established and is well underway.
- Recruitment and data collection has begun and participants are completing all study procedures.
- Data is being transferred across sites and several specimen shipments were sent to the metabolism and genetics cores.
- Data dissemination starts, one manuscript was published and several publications are underway.
- Tasks to complete for the next annual report include: (1) Continue to recruit and enroll subjects for the study. (2) Run study participants through all procedures. (3) Continue data collection and data management. (4) Analyze demographic data for enrolled participants for the purpose of matching controls with PTSD positive participants. (5) Continue to process and ascertain biomarkers. (6) Test the most promising biomarkers for validation and replication on a second cohort of 50 cases/50 controls. (7) Continue to ship samples to UCSF, Emory University, Drs. Marti Jett and Lee Hood for analysis. (8) Disseminate data via manuscripts and conferences.

CONCLUSION:

According to the Statement of Work, the goal of the implementation phase of this study is to:

1. Enroll and evaluate 200 OIF/OEF veterans (100 PTSD positive & 100 PTSD negative).
2. Complete all assessment measures for the enrolled participants
3. Complete Data entry for enrolled participants
4. Conduct biomarker acquisition procedures
5. Deliver Material to cores
6. Conduct preliminary analyses on sample

In year 4 of the project we exceeded the goals and milestones in the SOW by enrolling 86% of the required sample size, and meeting all the milestones for this project.

We will continue with validation and replication of the most promising biomarkers on a second sample size of 50 cases/50 controls, and data will be compared with the animal model.

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